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Metamorphosis of *Polycitor mutabilis* (Ascidiae compositae)

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In my previous paper (1942) a description was given of a new species of *Polycitor* from Japan together with some notes on its life-history. In the summer of 1942 I could observe the metamorphosis of that interesting ascidian. Reserving the detailed account for some future paper, I will give below a brief description of the metamorphosis. The results of some experiments I have made to elucidate the causes of metamorphosis will also be discussed.

DESCRIPTION OF THE LARVA

As is stated in my previous paper (1942), the breeding season of *Polycitor mutabilis* is July. Eggs and embryos in various stages of development may then usually be found in the atrial chamber of mature individuals.

To obtain a sufficient number of free-swimming larvae we adopted the following procedure. Fresh ripe colonies[1)] are cut each in four or five slices, then put in a small vessel containing fresh sea-water. In one hour or so, larvae come out from the colonies and swim about.

No diurnal or lunar periodicity in the liberation of larvae has been observed.

The swimming is characterized by a series of alternate periods of rest and activity as in most other ascidian larvae. The duration of the free-swimming state is relatively short, not exceeding 15 minutes. In

1 Ripe colonies are easily recognizable as such, for they contain many eggs and embryos, which are beautifully red in colour.

a case I have observed that the larva succeeded in getting itself fixed in 3 minutes.

The larva when first liberated measures about 2,5 mm in length, the tail being twice as long as the trunk. In the trunk we see three fixing papillae at the tip, arranged in a vertical row. Besides, we see the eye and the statolith on the dorsal side as two black spots. Further, we find many epidermal outgrowths around the papillae. The tail is, as in many compound ascidians, rotated about its longitudinal axis through 90° anticlockwise as seen from behind. The red colour of the trunk is due to the yolk-laden mass of endoderm that fills the central part of the body. The test of the trunk is at first entirely cell-free.

METAMORPHOSIS

To facilitate the observation larvae are reared attached to glass slides. A larva is taken up with a pipette and placed on an ordinary glass slide for microscopic use. It is then covered with a cover glass. The distance between the slide and the cover is regulated by a thin layer of vaseline put between them. The slide is then kept under running sea-water. The larva thus treated throws off its tail and transforms into a small ascidian just as in the natural condition.

As is well-known, the ascidian metamorphosis comprises two distinct processes, which run parallel to each other. The one is regressive, the other progressive. In the former process, larval organs, such as fixing papillae, tail, brain, etc., disintergrate. In the latter process the young ascidian is formed.

a) Regressive metamorphosis. Soon after the fixation the three fixing papillae begin to degenerate, and in 2 hours there is nothing left of them. At the same time the tail begins to degenerate also. In most ascidians the degenerating tail is resorbed in the trunk. In our species, as in *Distaplia magnilarva* described by Salensky (1893), the tail is simply thrown off from its base. When involution begins, the tail is shortened and, at the same time, coiled up in an irregular way, especially at the tip. Then a constriction appears at the base of the tail. Finally the axial organs break in two portions. Those lying before the constriction are resorbed in the trunk, whilst those lying behind are thrown off with the test. In both portions intensive phagocytosis is seen. In 2 days old larvae the debris of the degenerated tail is still visible in the trunk. The degeneration process attacks also the brain. But the sense organs persist for a longer period and in 7 days old larvae they are still recognizable as two black spots in the body-cavity.

b) Progressive metamorphosis. Hand in hand with regressive changes there occur also progressive changes and in 24 hours a young ascidian is formed. Of these progressive changes we take up here only the formation of epidermal vesicles. Shortly before or after the fixation many epidermal outgrowths appear at the chin. They become epidermal tubes. Finally, they are separated from the trunk and wander out in the test as small, closed vesicles. Their wall consists of a single layer of polygonal cells, which are ectodermal in origin. On one side, i.e. that side which lies most distally when separation occurs, the wall is thick and

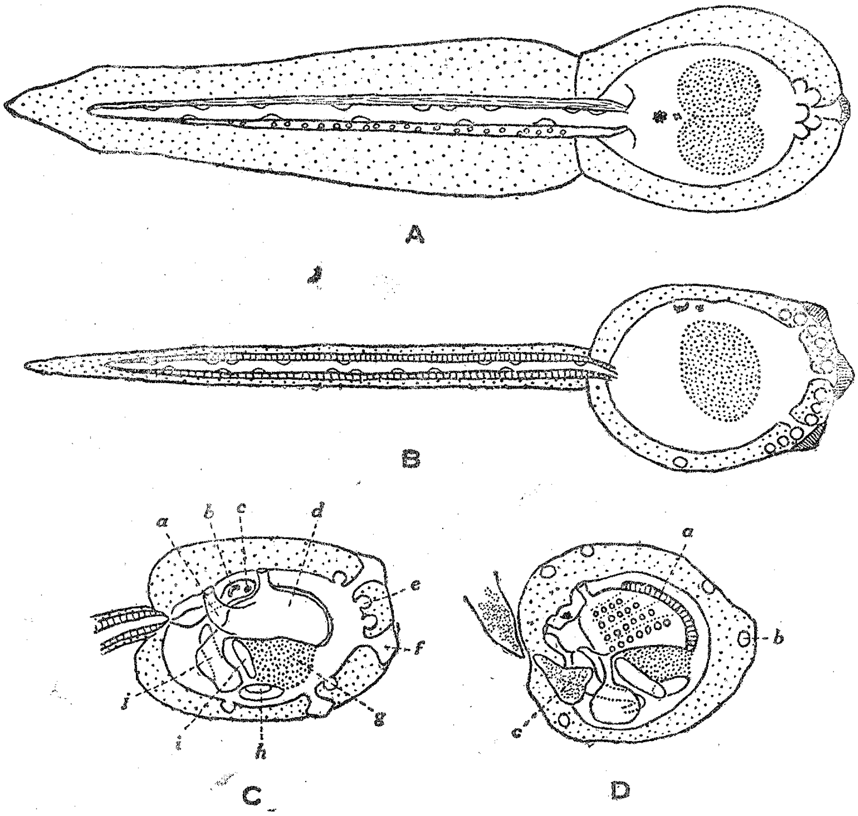

Fig. 1. A. Free-swimming larva, dorsal view. B. The same, lateral view. C. Internal structure of free-swimming larva. Reconstruction from sections. a. right pouch of atrium. b. eye, c. statolith, d. pharynx, e. epidermal outgrowth, f. fixing papilla, g. yolk-laden mass of endoderm, h. heart, i. epicardium, j. alimentary tract, D. Internal structure of one day old larva, Recontruction from sections. a. endostyle, b. epidermal vesicle. c. debris of the degenerated tail. ×40.

glandular, its cells being columnar instead of cubic as elsewhere. The interior of the vesicle, which is nothing more than an isolated portion

of the primary body cavity, does not contain mesodermal cells. The formation of such epidermal vesicles is not necessarily restricted to the preoral region, for at least one pair is formed from the ventral wall of the trunk a little behind the heart region.

The epidermal vesicles are not larval organs, for we find them also in the test of full-grown colonies. As for their function we can reckon *a priori* upon two possibilities. Either they serve as fixing processes or they serve as conveyers of nourishment from the body to the test cells.

Experiments

The experiments were designed with two objects in mind. The first was to investigate the relation of the degeneration of the fixing papillae to the involution of the tail. The second was to make clear the rôle of the involution of the tail in the process of metamorphosis.

In the first series of experiments we cut off the anterior end of the trunk, thus removing the fixing papillae. The result was as follows. The involution of the tail was suppressed, nevertheless the progressive metamorphosis took place and a complete young ascidian was formed.

In the second series of experiments we cut off the tail precociously. The result was that the progressive metamorphosis proceeded with just the same tempo as in the normal animal, and a young ascidian was formed.

From these results we may conclude that the regressive processes have nothing to do with the initiation of the progressive processes, while the degeneration of the fixing papillae and that of the tail, both belonging to regressive metamorphosis, are causally related to each other.

Our results agree quite well with those obtained by Zhinkin (1939a) in *Botryllus*. Compared with those obtained by E. Holmgren (1933) in *Clavelina*, ours are different in that in our species the progressive metamorphosis can take place without the preceding involution of the tail. In *Clavelina* the ascidian body degenerates, when the tail is amputated precociously. This, according to Holmgren, is attributable to the accumulation of some unknown histolytic sustances in the trunk, which in the normal development are given off to the tail to cause its degeneration (p. 205). In our species, even in the normal development, only a small fraction of the tail is resorbed in the trunk, in other words, the tail degeneration plays only a subordinate rôle in the progressive metamorphosis. This is perhaps responsible for the difference in behavior between *Clavelina* and *Polycitor*. To decide this and other questions more extensive experiments are needed.

CORRIGENDUM

Owing to the difficulty in determining the exact position of the heart in mature individuals, in living as well as preserved specimens, I have described the postabdomen erroneously as having neither epicardium nor heart (1942). Examination of the sections has revealed that the epicardium is extended half-way back in the postabdomen and at its end the heart is situated. But I do not think that this correction has any bearing upon the systematic position of the animal given in my previous paper.

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